

## Original article

# Quantification of peripheral blood CD34<sup>+</sup> cells prior to stem cell harvesting by leukapheresis: a single center experience



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## ABSTRACT

**Background:** Due to laboratory logistic issues, our center has traditionally scheduled peripheral blood stem cell harvests based on timing from the start of mobilization. This has proved to be useful in some cases, but also resulted in many fruitless harvests due to poor mobilization. In order to improve the efficiency of collections and compare the effectiveness of peripheral blood CD34<sup>+</sup> cells as a predictor with data from other reports, this study analyzed the implementation of this routine.

**Methods:** Peripheral blood and leukapheresis samples were quantified by flow cytometry and the association between these parameters was assessed.

**Results:** Sixty-six consecutive leukapheresis samples were collected from 34 patients after the collection of peripheral blood samples for CD34<sup>+</sup> quantification. A moderate positive correlation was observed between peripheral blood CD34<sup>+</sup> cell count and total CD34<sup>+</sup> cell count/kg ( $r = 0.596$ ;  $p$ -value  $< 0.001$ ). A multivariable regression model also confirmed this association and allowed the estimation that for every increase in five CD34<sup>+</sup> cells/ $\mu$ L in the peripheral blood, a mean increase of  $0.38 \times 10^6$  CD34<sup>+</sup> cells/kg could be predicted. Demographic characteristics, baseline comorbidities and mobilization regimen did not influence final CD34<sup>+</sup> cell count in this sample.

**Conclusions:** As observed in other centers, quantification of peripheral blood CD34<sup>+</sup> progenitor cells is a strong predictor of effectiveness to guide stem cell harvesting. Due to the results of this study, a modification in the peripheral blood stem cell harvesting logistics was implemented at our center in order to incorporate this routine.

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## Introduction

Hematopoietic stem cell transplantation (HSCT) is a treatment modality that allows the administration of high intensity chemotherapy (conditioning) without causing permanent myeloablation and the infusion of hematopoietic stem cells (both autologous or allogeneic).<sup>1</sup> Hematopoietic progenitor cells may be obtained either from bone marrow (BM), peripheral blood (PB) or umbilical cord blood (UCB).<sup>1</sup> The widespread use of bone marrow as a source of stem cells for the treatment of hematological, oncological, hereditary and immunological diseases, derives from more than a century of research.<sup>1</sup>

Peripheral cells are collected using an apheresis machine after being mobilized from the bone marrow to PB. Currently, this technique is applied in more than 90% of autologous bone marrow transplants (BMT) and in approximately 70% of allogeneic BMT.<sup>2</sup> These cells, known as peripheral blood stem cells (PBSCs), became the preferred source for autologous HSCT.<sup>3</sup> The advantages of PBSCs over BM stem cells in autologous settings include faster hematopoietic recovery, better immunological reconstitution and a relatively easy collection process.<sup>4</sup> The use of PBSC in allogeneic settings is still not the first choice due to its impact on the modulation of graft-versus-host disease (GVHD).<sup>4</sup>

Hematopoietic progenitor cells typically express the CD34 antigen on the cell membrane and this has been correlated with colony forming units in cell cultures, which is considered the gold standard for stem cell quantification. Furthermore, the quantification of CD34<sup>+</sup> cells by flow cytometry is widely used in the clinical practice as an indirect indicator of hematopoietic progenitor cells. Under normal conditions, CD34<sup>+</sup> cells in PB range from 0.01 to 0.05%;<sup>5</sup> in the BM, the concentration is usually less than 1% of normal mononuclear cells.<sup>5-8</sup> The number of progenitor cells to be infused to reach a proper hematopoietic recovery is still controversial,<sup>9,10</sup> however a minimum of  $2-5 \times 10^6$  CD34<sup>+</sup> cells/kg of body weight is required to achieve consistent engraftment.<sup>9,10</sup>

Classical strategies to mobilize PBSCs include the administration of hematopoietic growth factors such as the granulocyte colony-stimulating factor (G-CSF), filgrastim, which is the most used protocol in our setting. The use of G-CSF may cause side effects, such as bone pain, headache, and low-grade fever, although these symptoms rarely affect PBSC harvesting.<sup>11</sup> Other centers use different colony-stimulating factors, such as sargramostim and stem cell factor, as well as other adjuvant substances, such as plerixafor, and the traditional regimen generally combining chemotherapy (CT) with cyclophosphamide, and G-CSF.<sup>5,12-14</sup> PBSCs for autologous transplantation are usually collected by leukapheresis during hematological recovery after CT and/or during the administration of mobilizing agents.<sup>7</sup> However, the kinetics of the CD34<sup>+</sup> cell concentration in the PB is difficult to estimate and varies depending on the mobilization regimen used.<sup>7</sup>

Predictive factors for an effective harvesting have been broadly studied.<sup>15</sup> These include parameters obtained prior to the beginning of the procedure that influence the efficiency of harvesting CD34<sup>+</sup> cells.<sup>12,13,16</sup> The total leukocyte count,

number of monocytes and lymphocytes, and percentage of circulating immature cells of the granulocytic lineage have all been mentioned as possible predictive factors for apheresis collection.<sup>12,13,15,16</sup> Among these factors, the monitoring of PB CD34<sup>+</sup> cell concentrations by flow cytometry<sup>10,17</sup> has emerged as a reliable method to predict the success/failure rate of collections. The concentrations obtained by leukapheresis are directly correlated with the quantification of CD34<sup>+</sup> cells in PB,<sup>18-22</sup> however the final yield obtained in the apheresis product is, to some extent, variable. A cut-off for CD34<sup>+</sup> cell count of  $\geq 10$  to  $20 \times 10^3$ /mL is usually considered a reasonable cut-off value for an effective PBSC collection.<sup>14</sup>

Traditionally at the HCPA, PB stem cell harvesting was scheduled based on median days to CD34<sup>+</sup> peak cell concentration (at around 4-5 days of mobilization exclusively with filgrastim, or on the 11th day following a cyclophosphamide-based regimen). This routine was established due to laboratory limitations in respect to the availability of flow cytometry and proved adequate in a significant number of patients. However, in up to 20% of patients, this strategy resulted in futile procedures due to poor mobilization.

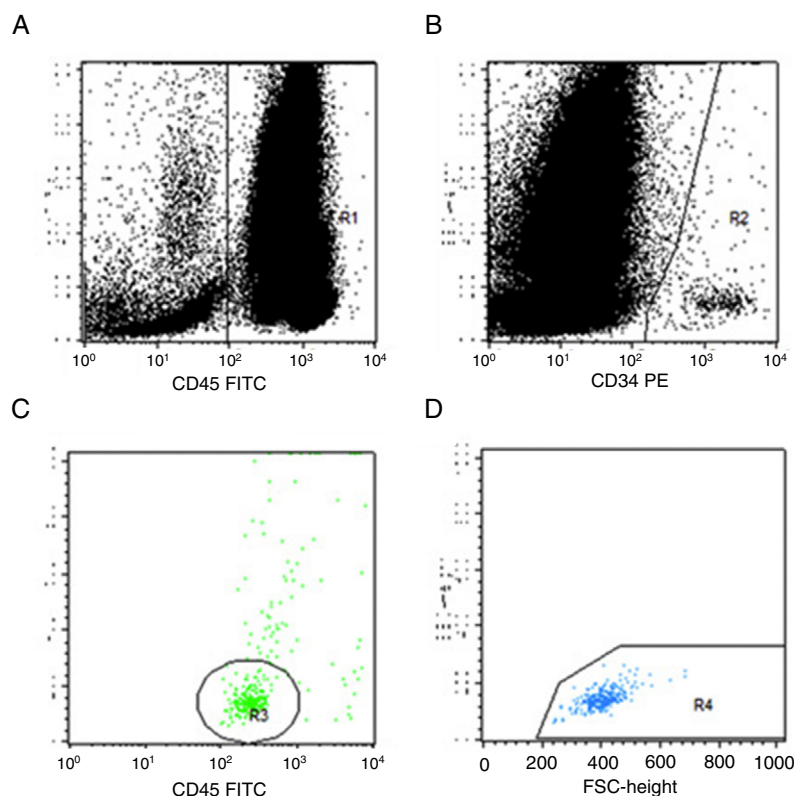
In order to validate a new routine and assure its effectiveness in preventing unnecessary procedures, a strategy was applied at the HCPA to collect a PB sample to quantify peripheral CD34<sup>+</sup> cells prior to the apheresis collection. This study describes the feasibility of quantifying CD34<sup>+</sup> cells in the PB prior to apheresis in order to improve the success rate of apheresis collections in a local sample of patients.

## Methods

This study was conducted in the Apheresis Unit and Clinical Pathology Department of the HCPA and was approved by the local Research Ethics Committee (number: 12-0326). All the enrolled patients signed written informed consent forms before participating in the research protocol. The study was conducted in accordance with the Helsinki Declaration as revised in 2008. All patients referred for autologous bone marrow transplantation were included irrespective of underlying disease and compliance with the mobilization protocol for apheresis. No exclusion criterion was applied.

Results for the quantification of CD34<sup>+</sup> cells in apheresis samples were obtained by the previously established routine assessment of PBSC collections. CD34<sup>+</sup> progenitor cells were quantified by flow cytometry using the International Society of Hemotherapy and Graft Engineering (ISHAGE) protocol.<sup>23</sup>

PB samples for CD34<sup>+</sup> cell counts were collected from each patient immediately before beginning leukapheresis. Samples were kept at room temperature (25 °C) for 1 h prior to CD34<sup>+</sup> quantification. PB CD34<sup>+</sup> cells were counted with a FACSCalibur flow cytometer<sup>®</sup> (Becton Dickinson, San Jose, CA, USA) using CD45 and CD34 clone 8G12 monoclonal antibodies (Becton Dickinson, San Jose, CA, USA) conjugated with the fluorochromes fluorescein isothiocyanate and phycoerythrin, respectively, according to the ISHAGE protocol to identify CD34<sup>+</sup> cells by flow cytometry. After the addition of monoclonal antibodies, cells were homogenized, incubated for 15 min at room temperature, and the red blood cells were



**Figure 1 – Gates strategy used according to recommendations of the International Society of Hemotherapy and Graft Engineering (ISHAGE). (A) Region positive for CD45 cells representing all leucocytes (R1 gate). (B) Positive for CD34 from immature cells (R2 gate). (C) Combined events of R1 and R2 with Gate R3 selecting CD45<sup>+</sup> low complexity cells. (D) Cells selected by FSC/SSC and the percentage of CD34<sup>+</sup> cells was determined (R4 gate).**

lysed and washed with phosphate buffered saline (PBS). Subsequently, cells were resuspended in PBS and counted by flow cytometry. Two hundred thousand CD45<sup>+</sup> events were acquired, in order to ensure the counting of at least 100 CD34<sup>+</sup> cells according to recommendations of the ISHAGE protocol (Figure 1). Results were obtained and were analyzed using the Cell Quest software (Becton Dickinson, San Jose, CA, USA).

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 20.0 (SPSS, Chicago, IL, USA). Variables were described depending on their distributions and normality was assessed with the Shapiro–Wilk test. Continuous variables were compared using the Pearson or Spearman correlation as indicated by their distributions. A linear mixed regression model was assembled to evaluate multiple variables of interest. A *p*-value < 0.05 was considered statistically significant.

## Results

Sixty-six consecutive leukapheresis samples from 34 patients were analyzed. The first leukapheresis was performed four days after the beginning of G-CSF mobilization. Some patients underwent an alternative regimen including CT and G-CSF. In these cases, samples were collected 11 days after the beginning of the treatment.

Patient baseline characteristics are shown in Table 1. The mean age was 39 years (range: 1–68 years), the proportion of males was 52.9% and the most prevalent indication for auto-transplantation was multiple myeloma (MM) (44.11%).

The percentage of CD34<sup>+</sup> cells was higher in apheresis compared to PB samples (0.15% vs. 0.03%, respectively; *p*-value < 0.001). Accordingly, the mean total leukocytes was also higher in apheresis compared to PB samples (155,794/μL vs. 35,563/μL, respectively; *p*-value < 0.001). This proves the capacity of the apheresis system in centrifugation and concentration of the patient's buffy coat.

Correlations between variables of interest are shown in Table 2. A moderate positive correlation was found between CD34<sup>+</sup> cells/μL from PB and the total PB CD34<sup>+</sup> cells/kg body weight/procedure (*r* = 0.596; *p*-value < 0.001; Figure 2). In an attempt to analyze sensitivity, the persistence of this association was evaluated while stratifying data for different baseline characteristics; this correlation remained significant (*p*-value < 0.01) for subgroups of gender, diagnosis and mobilization regimen.

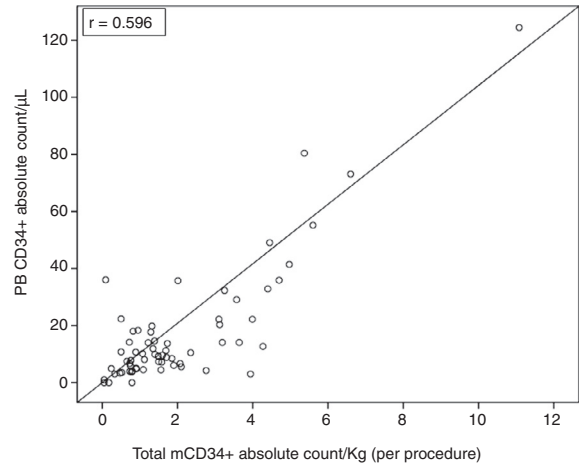
Finally, a multivariable model approach was used to evaluate different possible predictors of harvest results. In a forward stepwise manner, univariate models were assembled to exclude variables that were not significant considering a *p*-value < 0.2 (Table 3). The only predictor that persisted

**Table 1 – Characteristics of 66 peripheral blood cell harvests of 34 patients.**

Characteristic	Distribution
Gender (male) – n (%) <sup>a</sup>	18 (52.9)
Age – median (range) <sup>a</sup>	39 (1–68)
Diagnosis – n (%) <sup>a</sup>	
Multiple myeloma	15 (44.11)
Neuroblastoma	5 (14.7)
Non-Hodgkin lymphoma	4 (11.76)
Hodgkin lymphoma	4 (11.76)
Testicular neoplasm	3 (8.85)
Wilm’s tumor	2 (5.89)
Amyloidosis	1 (2.94)
Mobilization regimen – n (%) <sup>a</sup>	
G-CSF	33 (97.06)
Cyclophosphamide plus G-CSF	1 (2.94)
PB leukocyte count – mean (SD) <sup>b</sup>	35,563.38/ $\mu$ L (31,240.02–39,886.75)
PB CD34 <sup>+</sup> cell percentage – median (range) <sup>b</sup>	0.03% (0.0–0.27)
PB CD34 <sup>+</sup> absolute count – median (range) <sup>b</sup>	10.2/ $\mu$ L (0.0–124.4)
Leukapheresis leukocyte count – median (range) <sup>b</sup>	155,794/ $\mu$ L (29,860–507,760)
Leukapheresis CD34 <sup>+</sup> cell percentage – median (range) <sup>b</sup>	0.15% (0.02–0.79)
Leukapheresis CD34 <sup>+</sup> absolute count – median (range) <sup>b</sup>	156.9/ $\mu$ L (10.1–2601.1)
Final PB harvest yield (per procedure) – median (range) <sup>b</sup>	$1.4 \times 10^6$ CD34 <sup>+</sup> cells/kg (0.05–11.08)

PB: peripheral blood.  
<sup>a</sup> 34 patients.  
<sup>b</sup> 66 procedures.

through this stepwise exclusion was the PB CD34<sup>+</sup> cell count ( $p$ -value < 0.001) and according to the regression equation, each increase in five cells/ $\mu$ L resulted in a mean increase of  $0.38 \times 10^6$  CD34<sup>+</sup> cells/kg body weight/per procedure.



**Figure 2 – Correlation between absolute CD34<sup>+</sup> count/ $\mu$ L from peripheral blood and total absolute CD34<sup>+</sup> count/kg per procedure. Correlation between total absolute CD34<sup>+</sup> count/kg per procedure (x-axis) and absolute CD34<sup>+</sup> count/ $\mu$ L in peripheral blood samples (y-axis) ( $R = 0.596$ ;  $p$ -value < 0.001).**

**Discussion**

The CD34<sup>+</sup> cell count has been the standard method to quantify stem cells for decades now and its use to guide harvesting time has been concretely established.<sup>10,16</sup> The majority of studies showed a positive and reliable correlation between PB and apheresis CD34<sup>+</sup> cell content, which indicates that monitoring the PB is effective to predict harvesting results. In the present study, we found a moderate correlation similar to previously described reports.<sup>10,17</sup> This correlation was independent of gender, diagnosis or mobilization regimen. The MM patient subgroup showed the highest correlation coefficient

**Table 2 – Correlation analysis for quantitative measures of peripheral blood hematopoietic stem cell harvests (66 procedures).**

Variables correlated (total sample and stratified)	Correlation coefficient	p-Value	
PB CD34 <sup>+</sup> absolute count/ $\mu$ L	Total CD34 <sup>+</sup> cell count/kg (per procedure)	0.596 <sup>a</sup>	<0.001
Gender			
Male		0.605 <sup>a</sup>	<0.001
Female		0.588 <sup>a</sup>	<0.001
Diagnosis			
Multiple myeloma		0.723 <sup>a</sup>	<0.001
Other diseases		0.514 <sup>a</sup>	0.002
Mobilization regime			
G-CSF		0.573 <sup>a</sup>	<0.001
PB leukocyte count	PB CD34 <sup>+</sup> cell percentage	-0.077 <sup>a</sup>	0.541
Collection day (first, second and third)	PB CD34 <sup>+</sup> absolute count	-0.296 <sup>b</sup>	0.003
Leukapheresis leukocyte count	Final PB harvest yield (per procedure)	0.260 <sup>a</sup>	0.035

PB: peripheral blood; G-CSF: granulocyte colony-stimulating factor.

<sup>a</sup> Spearman Rho.

<sup>b</sup> Kendall Tau-b.

**Table 3 – Mixed model analysis for final PB harvest yield (per procedure) outcome (excluding univariate model variables not significant at  $p$ -value < 0.2).**

Variable	PB CD34 <sup>+</sup> absolute count (for each additional 5 cells/ $\mu$ L)
Mean outcome change	$0.38 \times 10^6$ CD34 <sup>+</sup> cells/kg body weight
95% confidence interval	$0.32$ – $0.44 \times 10^6$ CD34 <sup>+</sup> cells/kg body weight
$p$ -Value	<0.001

PB: peripheral blood.

The variables: gender, mobilization regime, diagnosis, collection day and peripheral blood leukocyte count were found non-significant and therefore were not included in the final model.

within the sample, according to their low expected failure rate.<sup>24,25</sup>

A negative correlation was seen between the total leukocyte count and the percentage of CD34<sup>+</sup> cells, indicating that the total leukocyte count should not be used as a predictor of effective harvesting. A negative correlation between the absolute CD34<sup>+</sup> count and collection day was also seen. The number of CD34<sup>+</sup> cells decreased with time even if the patient was still receiving G-CSF. Thus, the first collection had higher yields of CD34<sup>+</sup> cells.

Regression analysis allowed the estimation that for every increase of five CD34<sup>+</sup> cells/ $\mu$ L in PB, a mean increase in  $0.38 \times 10^6$  CD34<sup>+</sup> cells/kg body weight could be expected. Based on this result, a cut-off value of 15 cells/ $\mu$ L in the PB should be used for a minimum of  $1.0 \times 10^6$ /kg of body weight CD34<sup>+</sup> cells to be collected in one procedure ( $3 \times 0.38 = 1.14$ ). The implementation of this strategy in patient evaluation prior to harvesting reduced costs and extra procedures (e.g. higher doses of mobilizing factors and longer wait time for re-collection) performed on the patient.

Even taking into consideration the different alternatives of mobilization for transplantation, there are still many patients who do not achieve the minimum number of cells. This is due to known or unknown factors related to the primary condition of the patient or the mobilization regimen used. Szmigielska-Kaplon et al.<sup>26</sup> demonstrated that there are molecular changes that can be related to the difficulty of collecting the stem cells according to the primary disease. Accordingly, many patients do not achieve an ideal minimum number of collected CD34<sup>+</sup> cells for transplantation ( $1.5$ – $3 \times 10^6$ /kg body weight).<sup>27</sup> Sometimes different mobilization regimens are required based on the underlying disease and prior treatment. The G-CSF or G-CSF plus CT mobilization regimen is widely used,<sup>28</sup> but it has a relatively high failure rate of from 5 to 40%.<sup>29</sup> This failure occurs particularly in patients with non-Hodgkin lymphoma (NHL) with a failure rate of nearly 26%, while MM has a failure rate of 6%.<sup>24,25</sup> This may explain our finding that patients with MM have higher CD34<sup>+</sup> cell counts than patients with other diseases.

Pusic and Wuchter<sup>24,28</sup> analyzed 840 patients mobilized with CT and G-CSF. These authors demonstrated that the failure rate of mobilization decreased with the implementation of this treatment regimen (10.8%). Thus, CT combined with

growth factors may improve the mobilization of progenitor cells to the PB.

Plerixafor is another mobilizing agent with a synergistic effect when associated with G-CSF and may increase the number of CD34<sup>+</sup> cells by up to 3.8 times.<sup>28</sup> One disadvantage of plerixafor is that mobilization is rapid with its effect disappearing within 24 h. Moreover, it has a high cost, which hinders its use in our setting. In Europe, a study conducted with 508 patients (270 NHL, 54 Hodgkin's lymphoma and 256 MM) found that plerixafor was efficient in cell collection ( $>2 \times 10^6$ /kg body weight) in 64.8% of NHL patients, 81% of Hodgkin's lymphoma patients and 81.6% of MM patients.<sup>25</sup> Plerixafor has limitations and disadvantages, but it is a good alternative for patients with poor mobilization.

This study was limited by the sample size analyzed. Nonetheless, we believe that it is important to share the experience of our center, as it may be applied to other institutions to reduce collection failure. This strategy reduced costs and improved logistics. It is known that this methodology is already used comprehensively as standard routine, however regionally based data are lacking and it is important to compare this experience with that of other centers. The dual platform methodology could also have implicated some variability in the results.

## Conclusion

In conclusion, in a local population of patients, PB CD34<sup>+</sup> cell counts predicted PB stem cell harvests in large volume leukapheresis. Thus, the counting of PB CD34<sup>+</sup> progenitor cells should be systematically used to guide stem cell harvesting by leukapheresis. This variable reliably predicted the final yield of PBSCs. This strategy led to an increase in the success rate of apheresis collections making this procedure more efficient, lowering the costs, and consequently eliminating extra procedures of patients.

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## Conflicts of interest

The authors declare no conflicts of interest.

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